

## FREEING RABIES VIRUS OF THE NEUROALLERGENIC FACTOR FROM BRAIN TISSUE

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*Summary.* — A method of fixed rabies virus purification from infected sheep brains was proposed. It consisted of suspending the brain tissue in phosphate-buffered hypertonic (0.3 M) NaCl solution, shaking at 37 °C and low speed centrifugation at the same temperature. From 65 to 85% ballast proteins were removed, the neuroallergenic activity of the material was lost, but practically no losses of the virus occurred.

*Key words:* rabies virus; rabies vaccine; neuroallergenic activity

Anti-rabies vaccines prepared from infected animal brains are not fully innocuous. They contain brain tissue which can cause local allergic reactions, postvaccinal shock, neuralgia, neuritis, myelitis in the lumbar region with paresis of the muscles, Landry type ascending encephalomyelitis and postvaccinal encephalitis. The incidence of neurological complications caused, e.g., by the Hempt vaccine prepared from adult rabbit brains, is one per 1200—1700 vaccinees (Kuwert, 1977). The neuroparalytic accidents are less frequent with vaccines prepared from brain tissues of newborn animals.

It proved difficult to free antirabies vaccines from the neuroallergenic factor because the bond between rabies virus and brain cell components is firm and removal of the cell components usually is accompanied with a loss of most virus. Attempts have been made to purify rabies virus by treatment of brain tissues with benzene and calcium carbonate (Bell *et al.*, 1949); methanol (Tagaya *et al.*, 1959); freon (Diskina *et al.*, 1966; Pugach *et al.*, 1970); with the use of ion exchange resins (Thomas *et al.*, 1965; Turner and Kaplan, 1967) and by chromatography (Shokeir, 1968), but without satisfactory results.

Lavander (1970) succeeded to purify the virus without losses in its activity by isopycnic or zonal centrifugation in sucrose density gradients. This method is too complicated to be used in practice.

To obtain an anti-rabies vaccine from animal brains devoid of neuroallergenic activity, we developed a technically simple method which made it possible to remove from infected sheep brain suspensions about 80% of the

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ballast proteins along with the neuroallergenic factor; nearly no virus was lost.

**Virus.** Fixed rabies virus, strain "Moscow", representing the original Pasteur's strain after 3253 rabbit brain passages, was used in the form of brains from infected sheep up to 1 year old.

**Virus titration.** Tenfold dilutions in distilled water containing 2% calf serum were inoculated in 0.03 ml volumes into the brains of 7–10 g white mice. The latter were observed for 14 days and the titres were calculated by the Reed and Muench method.

**Virus purification.** A 20% suspension was prepared from infected sheep brain tissue in a warm (37 °C) hypertonic buffered solution containing 0.3 M NaCl, 0.005 M  $\text{KH}_2\text{PO}_4$  and 0.0035 M NaOH. The suspension was shaken for 1 hr at 37 °C and then centrifuged for 30 min at  $1350 \times g$  at the same temperature. The supernatant fluid was collected.

**Inactivation of virus.** Viral material in the form of a 10% suspension (with respect to the original weight of brain tissue) was mixed with an equal volume of a 15% sucrose solution in distilled water and then  $\beta$ -propiolactone (Sigma, U.S.A.) was added to a final concentration of 0.025%. After inactivation for 3 hr at 22 °C the material was kept for 48 hr at 4 °C until decay of  $\beta$ -propiolactone. The resulting vaccine was freeze-dried.

**Testing of the vaccine for absence of live virus.** The lyophilized vaccine was dissolved in distilled water to obtain a 5% suspension (with respect to the original weight of brain tissue) which was inoculated in 0.03 ml volumes into the brains of 10 mice. Ten days later five mice were killed; a 10% suspension from their brains was inoculated (0.03 ml) into the brains of 10 fresh mice which were kept under observation for 14 days.

**The neuroallergenic activity** of the purified vaccine was assayed in guinea pigs weighing 400–500 g as proposed by Svet-Moldavskij *et al.* (1965). The lyophilized vaccine was dissolved in distilled water to obtain a 50% suspension (with respect to the original weight of brain tissue) and mixed with an equal volume of complete Freund's adjuvant (Difco, U.S.A.). The mixture was inoculated in 0.2 ml volumes intradermally at 5 different areas of the skin on the belly of each animal.

The guinea pigs were observed for 40 days for symptoms of allergic encephalomyelitis. From a part of dead and surviving animals, the brains and spinal cords were removed for pathomorphological examination.

In parallel control experiments, an anti-rabies vaccine from whole sheep brain was used. The virus was inactivated according to Osidze *et al.* (1980): a 13.5% brain suspension was treated with 15% ethanol for 14 days at 37 °C and then lyophilized. The vaccine was checked for the absence of live virus by intracerebral inoculation of mice as described above.

**Protein content** was determined according to Lowry *et al.* (1951).

In the course of the purification procedure from infected sheep brains, the titres of rabies virus remained unchanged or decreased insignificantly. At the same time the amounts of non-viral proteins decreased by 66–86% (Table 1). The final purified virus vaccine representing a 5% brain tissue suspension (with respect to the original weight of the brain) contained less

**Table 1.** Virus titres and protein contents of 20% infected sheep brain suspensions before (I) and after (II) purification

		Experiment								
		1	2	3	4	5	6	7	8	9
Virus titre (log $\text{LD}_{50}/0.03$ ml)	I	6.5	7.5	6.25	7.1	7.5	6.7	6.0	7.25	6.12
	II	6.5	7.5	6.25	7.21	6.5	7.1	6.25	7.5	5.44
Protein contents mg/ml	I	34.9	35.0	28.0	21.2	32.0	28.5	24.8	30.8	24.3
	II	9.5	7.0	9.4	4.25	5.4	9.1	5.3	8.1	5.0

**Table 2. Testing in guinea pigs of the neuroallergenic activities of anti-rabies vaccines prepared from purified virus (I) and crude brain tissue (II)**

Exp. No.		No. of vaccine lots tested	Protein contents mg/ml	No. of guinea pigs tested/dead/survived	Allergic encephalomyelitis*
1	I	2	2.8-2.9	16/0/16	—
	II	1	12.0	6/5/1	—
2	I	2	2.2-2.45	14/0/14	—
	II	1	12.0	4/4/0	—
3	I	2	1.55-1.6	24/0/24	—
	II	1	11.75	13/8/5	—
4	I	1	1.75	9/0/9	6/0
	II	1	11.5	10/4/6	9/9
Total	I	7	1.55-2.9	63/0/63	6/0
	II	4	11.5-12.0	33/21/12	9/9

\*No. of animals tested/No. of animals with signs of allergic encephalomyelitis; — = not tested.

than 3 mg/ml protein as compared to 11.5-12.0 mg/ml in the control vaccine from whole brain tissue.

In the course of the purification procedure the brain suspension was freed of the neuroallergenic factor, as shown by the results of tests of 7 lots of vaccine prepared from purified virus (Table 2). Four lots of control vaccine from whole sheep brain tissue that contained no live virus were examined in parallel.

All 63 guinea pigs injected intradermally with the purified vaccine mixed with complete Freund's adjuvant survived. After 40 days of observation, six of the animals were subjected to pathomorphological examination. Morphological signs of allergic encephalomyelitis were not found.

On the other hand, 21 of 33 (63.6%) guinea pigs injected in parallel with the control vaccine from whole sheep brain tissue died with a typical clinical picture of allergic encephalomyelitis. Pathomorphological examination of the brains from 3 dead animals and 6 survivors revealed changes characteristic of allergic encephalomyelitis in all guinea pigs examined.

In spite of the numerous attempts at freeing rabies virus-containing brain suspensions of ballast substances, no method has yet been introduced into the practice of anti-rabies vaccine production. Our procedure of brain suspension treatment removed about 80% of non-viral proteins at practically no loss of virus. The method is technically simple and requires no complicated equipment. It consists of suspending brain tissue in a warm (37 °C) phosphate-buffered hypertonic NaCl solution, shaking and low-speed centrifugation. Apparently, in the hypertonic solution at 37 °C rabies virus was eluted from brain tissue fragments that were subsequently removed by centrifugation.

According to World Health Organization requirements, an anti-rabies vaccine is considered devoid of neuroallergenic activity if a 20% tissue suspension mixed with an equal volume of complete Freund's adjuvant

does not cause allergic encephalomyelitis on intradermal inoculation of 0.3 ml volumes in guinea pigs (Gispen, 1973). In the present study we prepared from the lyophilized vaccine a 50% suspension (with respect to the original weight of brain tissue) and after mixing with adjuvant we injected guinea pigs with 1 ml volumes each. Testing under such conditions of 7 lots of vaccine from purified virus showed them free of neuroallergic activity. Four lots of a control vaccine prepared in the same laboratory from crude sheep brain tissue caused under the same conditions death in more than 60% of the animals (according to WHO requirements, signs — at least morphological — of allergic encephalomyelitis should appear in at least 50% of control animals). These results allow the conclusion that the proposed method of rabies virus purification yields a preparation fulfilling the requirements posed on anti-rabies vaccines devoid of neuroallergic activity.

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